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PURIFICATION OF ENTEROTOXINS OF 'ESCHERICHIA COLI'.(U)
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18. SUPPLEMENTARY NOTES

Presented at the International Pharmacology Conference, Helsinki, Finland, July, 1975. A manuscript describing the progress and results of this work is under preperation.

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Affinity chromatography, Adenylate cyclase, Binding assay, Cholera toxin, Epidermal growth factor, Human fibroblasts, Escherichia coli, enterotoxin.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Affinity chromatography has been used to isolate and purify the diarrhea producing toxin from enteropathogenic E. coli culture media. Two simple, rapid and sensitive assay methods are devised to accurately quantitate the toxigenic material from crude culture media.

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FINAL *PROGRESS REPORT*

Contract #: DAMD17-75-C-5031

Duration: 4/1/75 thru 9/30/75

Title: Purification of Enterotoxins of Esherichia coli

Contractor: *The Johns Hopkins University*
Baltimore, Maryland 21205

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PROGRESS REPORT
Indu Parikh, Ph.D.

The general objective of this project was to purify the enterotoxin of Escherichia coli with the aim of studying its mechanism of action. The understanding of mechanism of action of this enterotoxin may provide knowledge for the development of rational and effective therapeutic measures for diarrheal disorders associated with this toxigenic agent.

The primary aim of the project consisted of devising methods of purification of E. coli enterotoxin based on the principle of affinity chromatography. As the interaction of this enterotoxin with cell surface gangliosides is specific and non-covalent, it was proposed to prepare various ganglioside - agarose affinity adsorbents. The following three types were prepared using bovine brain ganglioside (type III, Sigma):

1. Ganglioside-BSA-Agarose
2. Ganglioside-Poly-Lys-poly-Ala-Agarose
3. Ganglioside-diaminodipropylamine-Agarose

The substitution of the gangliosides on each adsorbent was between 2 to 5 umoles per ml of settled gel. The three affinity adsorbents were carefully tested for their usefulness in the purification of E. coli enterotoxin. The crude hyophilized culture filtrates of the pathogenic strain 408-3 was the source of this toxin. The bacteria were grown in 4 liter batches by the standard published procedures.

The affinity chromatographic experiments were carried out in small 1 ml bed volume columns. Approximately 10 mg proteins of the crude culture filtrate were applied to each of the three columns equilibrated with 25 mM Tris, pH 7.4. Almost 100% of the toxigenic activity was adsorbed on each of the affinity columns. The columns were then washed extensively with 25 mM Tris, pH 7.4 and eluted with various buffers containing either 2-5 M guanidine-HCl, pH 2 to 7, 2-8 M urea, various concentrations of variety of chaotropic agents, 0.05 M acetic acid or 0.05 M $NaHCO_3$. None of the above mentioned eluting agents were successful except the last one for elution of biologically active toxigenic material. Determination of specific activity of the bicarbonate eluted toxin revealed very little final purification (approx. 10-fold purification). All three of the above mentioned affinity adsorbents were similar in their relative efficiency in purification of the E. coli enterotoxin. Although the recovery of the toxin was impressive (more than 60%), all of the affinity adsorbents in combination with the bicarbonate elution technique were not promising for effective purification of E. coli enterotoxin. Further study with ganglioside-agarose adsorbents was discontinued.

Our next approach was to explore the use of anti-cholera toxin antibody, covalently immobilized on agarose matrix, as the affinity adsorbent for the purification of E. coli enterotoxin. Since 1969, it is known that antibodies raised against purified cholera toxin strongly cross react with

E. coli enterotoxin. Three rabbits were immunized with purified cholera toxin by standard procedures. The IgG fraction from the rabbit sera was isolated by ammonium sulfate precipitation. The partially purified IgG fraction was coupled to CNBr activated agarose. The substitution of the antibody on the agarose was about 8 mg per ml of settled gel. The affinity adsorbent thus prepared was used in the following experiments.

A column of five ml bed volume was packed with the affinity adsorbent described above and an aliquote of crude lyophilized culture medium containing 50 mg proteins was loaded onto the column previously equilibrated with Krebs-Ringer bicarbonate buffer. The toxigenic material was almost quantitatively adsorbed. In these experiments as in the case of the ganglioside-agarose adsorbents described above, none of the eluting techniques were promising except the bicarbonate buffer. The elution with 0.05 M $NaHCO_3$ was performed at 4° and resulted in a single elution peak containing all the toxigenic activities. Determination of specific activity (by our tissue culture method) indicated a purification of approximately 400-fold though with an overall yield of about 10%. The final yield of the purified material is too small to undertake large scale purification of this toxin. The degree of purification is also not yet that great to warrant direct application of this method for the purification of the E. coli enterotoxin.

Further experimentation in this direction is still being perused at our new location.

No publication or patent of any kind has resulted from this work.

The principal investigator is extremely thankful to the U. S. Army Medical Research and Development Command for their cooperation in this project.

Indu Parikh, Ph.D.

February 27, 1976